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14. ABSTRACT

Ovarian cancer is the most fatal gynecological malignancy. The understanding of the early molecular events leading to ovarian cancer is important for the development of strategies for early detection and prevention. We have demonstrated that DMBAinduced mutagenesis in the rat ovary, combined with gonadotropin hormone-mediated enhanced mitogenesis of the ovarian surface epithelium, produces lesions ranging from preneoplastic, early neoplastic to advanced ovarian tumors, resembling human disease. The goal of this project was to use the DMBA-gonadotropin animal model to study molecular mechanisms underlying ovarian oncogenesis and to conduct a preclinical chemoprevention trial. The original specific aims of the study were: 1) Determine the molecular genetic mechanisms underlying ovarian oncogenesis in the rat DMBA/gonadotropin model of ovarian cancer; 2) Determine the efficacy of the COX-1 inhibitor SC-560 to prevent the appearance and/or progression of DMBA-induced ovarian lesions; and 3) Study the in vivo mechanisms of the putative chemopreventive action of COX-1 inhibition. However, due to change of Principal Investigator in the last year of the study, the original research plan has been modified. Since the animal protocol pertaining to this project has been closed and the proposed chemoprevention trial in rats has not been initiated, only aim 1 is being carried out.

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MECHANISMS AND CHEMOPREVENTION OF OVARIAN CARCINOGENESIS
FINAL PROGRESS REPORT

INTRODUCTION

Ovarian cancer is the most fatal gynecological malignancy because of its asymptomatic development and frequent diagnosis at an advanced stage. The understanding of the early molecular events leading to the disease is important for the development of strategies for its early diagnosis and prevention, which could improve patient survival and quality of life. We have demonstrated that DMBA-induced mutagenesis in the rat ovary, in combination with gonadotropin hormone-mediated enhanced mitogenesis of the ovarian surface epithelium, produces lesions ranging from preneoplastic, early neoplastic to advanced ovarian tumors, which resemble human disease. The goal of this research project was to use the DMBA-gonadotropin animal model to study the molecular mechanisms underlying ovarian oncogenesis and to conduct a preclinical trial for its chemoprevention. The original specific aims of the study were:

1) Determine the molecular genetic mechanisms underlying ovarian oncogenesis in the rat DMBA/gonadotropin model of ovarian cancer

2) Determine the efficacy of the COX-1 inhibitor SC-560 to prevent the appearance and/or progression of DMBA-induced ovarian lesions

3) Study the *in vivo* mechanisms of the putative chemopreventive action of COX-1 inhibition

However, due to change of Principal Investigator (PI) in the last year of the study, the original research plan has been modified. Since the animal protocol pertaining to this project has been closed and the proposed chemoprevention trial in rats has not been initiated, only specific aim 1 is being carried out.

BODY

During the course of the project supported by this DoD-CDMRP grant, the following progress has been achieved along the proposed aims of the study:

1) Determine the molecular genetic mechanisms underlying ovarian oncogenesis in the rat DMBA/gonadotropin model of ovarian cancer. During the first year of support a large number of DMBA-induced ovarian lesions were generated in the rat at different stages of neoplastic development that would ensure statistical power and significance of the findings from their molecular classification and characterization. Using funds provided by the Fox Chase Cancer Center (FCCC) NCI Ovarian Cancer SPORE Grant, a two-phase carcinogenesis experiment was initiated at the end of 2003, in which 160 female 6-week old virgin female Sprague-Dawley rats were subjected to bilateral survival surgery to the ovaries. Animals were divided into four arms and treated: a) Control groups **a1** (20 animals, no hormones) and **a2** (20 animals, with hormones): beeswax-impregnated surgical sutures were implanted in the portion of each ovary that is contra-lateral to the fallopian tube; b) DMBA-/+hormone group (total 100 animals) **b1** DMBA/beeswax-impregnated surgical sutures were implanted bilaterally in the ovaries of the animals as above and **b2**. Two months following the surgical procedure, rats in group **a2** and **b2** were subjected to 4 cycles of sequential administration of hormones PMSG and hCG. These procedures are described in the Experimental Design and Methods section of our grant proposal and in our 2004 publication (1). All treated animals were maintained for one year from the survival surgical procedure, or until disease development and animal distress became

evident. Rats were sacrificed according to the initiation of treatment, in December 2004 and January 2005, following the Institutional Animal Care and Use Committee (IACUC) approved guidelines.

All of the ovaries were harvested and fixed in 70% ethanol at 4°C for 18hr, paraffin processed through a 12-hour cycle with a Tissue-Tek VIP 5 (Sakura) vacuum infiltration processor, and then paraffin embedded with a Histo-Center II (Fischer Scientific) embedding station. Three 5µm-sections, approximately 50µm apart of each other were obtained from the two end-portions of each ovary, stained with H&E and subjected to histopathological evaluation.

Table 1 below indicates the incidence of ovarian lesions observed in the four experimental arms, subdivided into 3 subgroups (nonneoplastic, putative precursors and neoplastic). This experiment was performed to verify the potential promoting role of gonadotropin hormones in ovarian cancer development, and to generate sufficient numbers of ovarian lesions for molecular characterization and elucidation of the mechanisms behind their development. Based on the observed statistically significant differences in lesion incidence between arms **a1** and **a2**, and **b1** and **b2** (Table 2), we suggest that gonadotropin hormones play a major role in the promotion of ovarian cancer.

Table 1. DMBA ovarian carcinogenesis with gonadotropin co-treatment

Experimental Arm	*per ovary*				*per animal*			
	No Lesions	Non-Neoplastic Lesions	Putative Pre-Neoplastic Lesions	Neoplastic Lesions	No Lesions	Non-Neoplastic Lesions	Putative Pre-Neoplastic Lesions	Neoplastic Lesions
a1 - Surgery only (20 animals) %	37.5	40.0	22.5	0.0	0.0	70.0	30.0	0.0
a2 - Surgery +Hormones (19 animals) %	20.8	21.1	58.1	0.0	0.0	26.1	73.9	0.0
b1 - DMBA (47 animals) %	15.7	20.5	62.8	1.0	6.3	13.0	78.7	2.1
b2 - DMBA+Hormones (45 animals) %	1.1	15.4	75.8	7.7	0.0	8.8	75.8	15.4

Table 2. Statistical significance of differences in lesion incidence induced by gonadotropin co-treatment (* - determined by γ-square and/or Fisher's exact tests)

Comparison*	Site of the lesions	P-value
Surgery vs. Surgery+Hormones	Ovary	0.0061
	Animal	0.0064
DMBA vs. DMBA+Hormones	Ovary	0.0002
	Animal	0.0422

Based on the histopathological characteristics and stage of neoplastic development, ovarian lesions detected in **b2** ovaries were subdivided into 7 categories: 1) normal ovarian surface epithelial (OSE) cells (>35 samples); 2) reti ovarii hyperplasia (12 lesions); 3) bursal and

OSE flat hyperplasia (28 lesions); 4) bursal and OSE papillary hyperplasia (34 lesions); 5) inclusion serous cyst with papillary hyperplasia (40 lesions); 7) non-invasive carcinoma (3 lesions); 7) invasive adenocarcinoma (5 lesions). Normal appearing OSE cells obtained from ovaries of **a1** (>15 samples) and **a2** (>15 samples) animals generated two additional sample (control) categories.

A similar report has most recently demonstrated that rats treated with systemic estrogen and local ovarian DMBA administration, simultaneously develop preneoplastic and neoplastic lesions in the breast and ovary (2). The same criteria was used to evaluate progression toward ovarian cancer as in our study, namely putative ovarian preneoplastic changes such as inclusion cysts, epithelial hyperplasia, papilloma and stromal hyperplasia.

Molecular characterization of the rat DMBA/gonadotropin-induced ovarian lesions

To determine whether, similar to human disease (3), COX-1 and/or COX-2 expression/activation is associated with ovarian neoplastic development in this animal model, we initiated collaboration with Dr. S. K. Dey at Vanderbilt University Medical Center. Histological slides were prepared from tissue sections obtained from formalin-fixed paraffin-embedded (FFPE) rat ovaries treated with DMBA or DMBA/hormones and containing putative preneoplastic (7 samples) or neoplastic lesions (5 samples). Each slide also contained a tissue section from the corresponding contra-lateral, control ovary. Individual slides, sent to Dr. Dey, were subjected to immunohistochemical (IHC) analysis for COX-1 or COX-2 expression. Elevated expression of both enzymes was observed in the majority of analyzed putative preneoplastic lesions and all neoplastic lesions regardless of progression. Neither protein was detectable in the OSE of normal (control) ovaries. Even though in most cases, the expression level of COX-1 was higher than that of COX-2, the data implied a strong association of both enzymes with ovarian cancer development in this model. Figure 1 shows examples of changes in

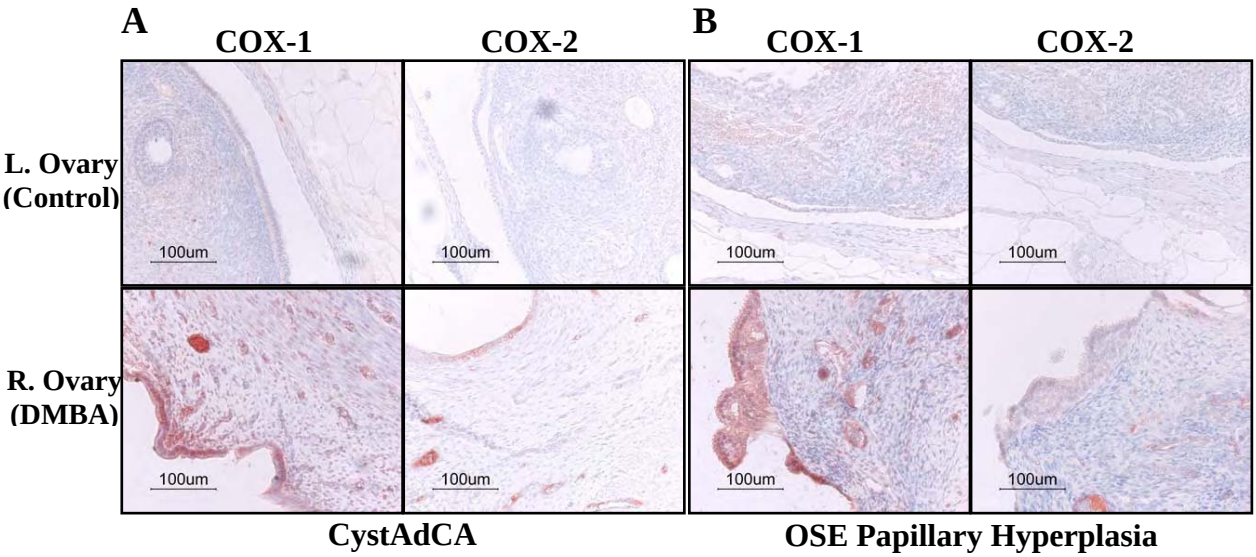

Figure 1. IHC staining for COX-1 (left half-panel A and B) and COX-2 (right half-panel A and B) protein expression in rat ovaries: Left (L. Ovary) untreated control (top panels) and Right (R. Ovary) DMBA-treated (lower panels). **A.** Cystadenocarcinoma; **B.** Surface epithelial papillary hyperplasia. Sections of left and right ovary from the same animal were mounted on the same slide and subjected to IHC at identical conditions. Pictures of each pair of sections per slide were taken at identical brightness/contrast settings.

COX-1/2 expression. These results are interesting, and though they support our original proposal for the pre-clinical testing of a COX-2 specific inhibitor (celecoxib) (see **2.** below), they also suggest that a COX-1 specific inhibitor (such as SC-560, Cayman Chemical Co) may be more effective as an agent for chemoprevention of ovarian cancer. The results also warrant further analysis of additional ovarian lesions, both putative preneoplastic and neoplastic, in order to evaluate the prevalence of the observed changes in COX-1/2 expression, and whether they are also present in putative preneoplastic lesions induced by gonadotropin hormone treatment alone.

We have previously performed a global, microarray-based gene expression analysis of human ovarian tumors and normal human ovarian surface epithelia (non-cultured or short-term cultured). Among the genes identified with differential expression between different types of tumors and normal OSE, the most interesting was the NF-κB regulator gene A20. While this gene was found expressed at moderate to high levels in the normal OSE, its expression was undetectable in all tested tumors, irrespective of their histological subtype or neoplastic stage (Fig. 2). This result suggests that A20 plays a confounding role in the development of ovarian carcinomas and could potentially play such a role in the DMBA/gonadotropin model. A20 is an enzyme with dual ubiquitination and de-ubiquitination activities and plays an important role as a switch between activation and inactivation of the NF-κB survival transcription factor (4, 5). While A20 facilitates the coupling of cytokine and other receptor signals to the IKK signalosome complex through RIP and other MAP3Ks, it is also essential for termination of the same signals and inhibition of a persistent NF-κB activation. The persistent, elevated activation of NF-κB has been associated with the malignant progression and development of resistance to cytotoxic treatment of many types of tumors. Therefore, loss of A20 in ovarian cancer may be one of the underlying mechanisms and a very important target for the design of new strategies for prevention and treatment of the disease. In support of this observation, the preliminary results obtained from a phase I trial of the proteasome inhibitor bortezomib in combination with platinum agents (carboplatin) for overcoming the development of chemoresistance of ovarian cancer patients are encouraging (6). Based on our results from the analysis of human normal OSE, we suggest that A20 would also be expressed at moderate levels in the normal rat OSE. Though the examination of expression status of A20 in the normal rat OSE and in DMBA/hormone-induced lesions at different stages of neoplasia by real-time qRT-PCR was originally planned, the analysis has not been initiated.

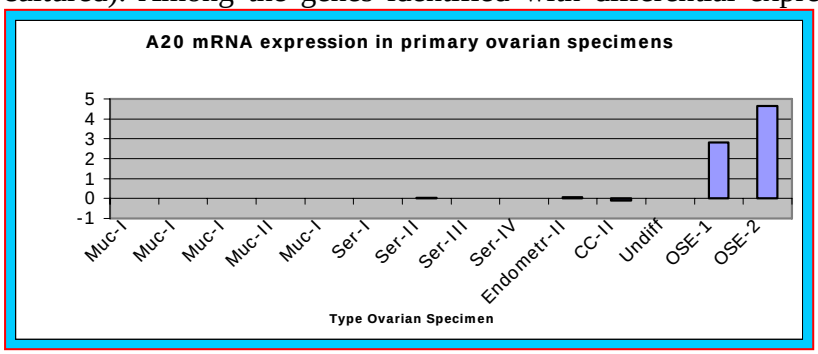

Figure 2. Microarray-determined A20 mRNA expression in primary human ovarian cancer specimens of different histological subtype and malignant stage, and in normal human OSE (OSE-1: average of 4 short-term cultures; OSE-2: average of 2 non-cultured samples). Data was confirmed by real-time qRT-PCR analysis (data not shown)

Genomic analysis of DMBA/gonadotropin-induced rat ovarian lesions

With the guidance of our collaborator, pathologist Dr. Klein-Szanto, we have achieved a complete histopathological examination of 262 ovaries harvested from 131 animals included in the 4 arms of the carcinogenesis experiment described above. This allowed the identification of ovaries that contain different types of lesions and the selection of lesions for the purpose of this

study according to their classification, as illustrated above. In a streamline fashion, ovaries selected for a certain type of lesion were then subjected to further processing in preparation for genomic analysis. In order to better preserve the quality of RNA, ethanol-fixed paraffin-embedded (EFPE) ovarian tissue blocks were kept at 4°C at all times. Depending on the size of lesion and its epithelial cell component, 4-6 5μm-sections were generated from the portion of the organ adjacent to the corresponding H&E sections and either stored at –80°C until they were subjected to laser-capture microdissection (LCM) or processed immediately. Prior to proceeding with laborious microdissections, the quality of isolated RNA was checked on tissue scrapes, using the Agilent 2100 Bioanalyzer and samples with unacceptable quality were excluded from the analysis. Ovarian tissue sections were stained with HistoGene LCM Staining Kit (Arcturus), and 2,000-5,000 OSE cells were collected on CapSure HS LCM Caps using either PixCell II or AutoPix LCM Systems (Arcturus).

It has been reported that a considerable variation in the microarray data is incorporated when different sets of arrays are used to compare specimens in a single experiment. This is an issue that may have a significant impact on the reproducibility and reliability of the microarray data generated for the purpose of our research. To avoid this, and since the preparation of tissue specimens, purification and amplification of RNA and quality testing are the rate-limiting procedures, we are processing all lesion samples to the point that we could carry out all hybridizations serially within a short period of time and with the same lot of microarrays. Therefore, we have stored all samples procured by LCM at –80°C and will proceed with the analysis from this point.

Here we would like to emphasize that in February of 2007 the PI status on the project has changed. Dr. Patriotis had left FCCC, and Dr. Cvetkovic, who had no prior involvement in this project, took over to finish up the study. Tissue samples generated along the lines of this DoD-funded research were transferred to the new laboratory. Some of them were already subjected to LCM, and some were not, in which case we completed the cell procurement. However, the problem with these samples is that they were fixed by an alternative method, using ethanol, and paraffin embedding, while the golden standard for molecular analyses are frozen tissue specimens (7, 8). The rationale behind ethanol fixation was to preserve tissue architecture and cellular morphology of the rat ovary, while allowing for the recovery of good quality RNA from microdissected cells. Despite the loss in morphologic quality in frozen sections, especially in non-cover-slipped slides for LCM, RNA quality is generally better than that of RNA from ethanol- or formalin-fixed cells (9). Moreover, the Arcturus LCM systems that were initially used to procure biological samples for this study have in the meantime undergone substantial technical improvements. The new generations of platforms, the upgraded manual PixCell II, and the automated Veritas, now have features that allow for superior visualization of cellular morphology, irrespective of the tissue fixation method, compared to previous generation PixCell II and AutoPix systems.

Though others have successfully recovered RNA from EFPE human and animal tissues sufficient for downstream molecular profiling studies (10, 11), we wanted to check the quality and amplifiability of RNA from DMBA/hormone-induced rat ovarian lesions on several levels prior to microarray analysis. We have consulted with the application scientists at Arcturus on how to approach this issue. Since the company makes kits designed exclusively for extraction of RNA from frozen (PicoPure RNA Isolation Kit) or FFPE tissues (Paradise Reagent System), we needed to determine which one would be more appropriate for EFPE samples. In addition to these, two other kits were included in the test, Recover All Total Nucleic Acid Isolation Kit (Ambion) and Optimum FFPE RNA Isolation Kit (Amgen). Two randomly picked EFPE rat tissue samples where cut onto 4 slides, and each one was scraped off and used for RNA

extraction with one of the 4 kits. The quantification and integrity determination of isolated RNA were carried out by micro fluidic electrophoresis on Agilent 2100 Bioanalyzer using the RNA 6000 Pico LabChip Kit (Agilent Technologies). Additional sample quality assessment was done by qRT-PCR protocol developed by Arcturus that uses 3' and 5' primer sets to amplify a portion of the beta-actin gene. The 3'/5' ratio evaluates the abundance of the average beta-actin cDNA from the 3' end compared to the abundance of a 5' sequence using the quantified PCR yields of each amplicon. If most of the cDNA contains both the 3' and 5'sequence target, the ratio of the PCR product for 3'/5' is close to 1. As the RNA starts exhibiting some level of degradation, the 3'/5' ratio tends to become greater than 1. Depending on the ratio, an estimation of the RNA quality can be made. A suggested cut-off would be ≤20. All of our samples were in the optimal 3'/5' ratio range from 3-11. There was no significant difference between the 4 kits, and therefore we decided to use the PicoPure RNA Isolation Kit.

Total RNA was isolated from the microdissected cells yielding ~5ng of total RNA per sample. RNA quantification and integrity assessment were carried out on the Bioanalyzer. Total RNA was then subjected to amplification using Low RNA Input Linear Amplification Kit (Agilent) with a modification in the protocol. In the first round of amplification, bacteriophage T7 RNA polymerase promoter sequence is incorporated into the synthesized first cDNA strand. Next, exogenous primers are added to the second-strand reaction in order to make ds-cDNA. In-vitro transcription is then performed using T7 RNA polymerase. After the first round of amplification, purified aRNA is converted into ds-cDNA, which is the template for a second round of amplification. Following two rounds of RNA amplification, the quality of the resulting amplified RNA products was checked on the Bioanalyzer. One of the primary limitations of microarray analysis is large amount of labeled input RNA (5 to 10 μg) required for hybridization. Therefore, small quantities of starting LCM material need to be amplified and high correlation between the expression profile of amplified and non-amplified samples should be maintained. In our hands approximately 5ng of total RNA is amplified 1000x and the ensuing 5μg are inputed into hybridization reaction.

Although previous annual reports have indicated the intent to use the Affymetrix GeneChip system for the genomic analysis of rat ovarian lesions, due to change of PI, limited time frame and resources, as well as cost-effectiveness of the Agilent platform, the decision has been made to utilize Whole Rat Genome Oligo Microarray Kit (Agilent) instead. This platform is now available at the Fox Chase Cancer Center DNA Microarray Facility.

In our microarray analysis of the rat ovarian lesions, we will examine the status of genes that are known targets of DMBA mutations, as well as genes that may specifically apply to human ovarian cancer, such as p53, K-Ras, and c-Myc, ER and PR receptors, HER2/neu, Akt2, Bcl-2, Bcl-x and Bax. Moreover, we expect to identify genes whose changes in expression are associated with increased ovarian lesion severity and malignant progression, from nonneoplastic and preneoplastic to neoplastic. The apparent OSE cell origin of DMBA-induced tumors (12), make this model not only convenient, but also relevant to disease in women and perhaps valid for testing of new prevention agents.

Microarray data will be analyzed to identify genes differentially expressed across lesion categories. Genes, whose intensity has a large coefficient of variation (CV>2.5) for each of the categories, estimated from at least 7 lesions per category from two replicate array measurements, will be discarded, according to Schena et al. (13). This way, a gene will be tested for differential expression only if its data shows sufficient reliability within lesion categories. One-way analysis of variance (ANOVA) will be conducted using the natural log of gene expression as the dependent variable and the indicator variable identifying lesion category as the treatment factor. The F statistic for treatment effect will be the criterion to assess overall differences in gene

expression across the lesion categories. Pairwise comparisons will be conducted to determine if the gene exhibits differential expression across each pair of lesion categories. Once differentially expressed genes have been identified, cluster analysis, based on the Pearson correlation coefficient, will be conducted to identify genes that share a similar pattern of expression across lesion categories (14). Based on previous studies, we are 90% confident that natural log expression for any one gene will have a SD no greater than 0.4. A Monte Carlo simulation study indicated that at least 7 lesions per category should be included so that genes that are at least 2-fold differentially expressed will be identified with 80% power at the 5% significance.

A tentative timetable for the execution of the remaining experiments during the fourth year on the project has been modified. At the time of the preparation of the final progress report, microarray analysis has been performed on the first 4 samples from the preneoplastic lesion category. On each of the array slides, the Rat Universal Reference Total RNA (Clontech) was hybridized against the sample. We will analyze 8 samples from 3 categories, nonneoplastic, putative preneoplastic and neoplastic, since we observed 8 neoplastic lesions (bonafide tumors) among all 4 experimental arms. As the obtained array images from the first 4 samples are of sufficient quality, we are proceeding with the analysis of additional samples. We are hopeful to complete the analysis in a brief period of time. Acquired array images will be processed to extract gene intensities, and the Bioinformatics Facility at FCCC will carry out statistical analysis and mining of data as described above.

2) Determine the efficacy of the COX-1 inhibitor SC-560 to prevent the appearance and/or progression of DMBA-induced ovarian lesions. The goal of specific aim 2 was to determine a reasonable choice of putative chemopreventive agent for a preclinical chemoprevention trial using the DMBA/hormone animal model of ovarian cancer, developed and characterized by us. The original goal of the proposed chemoprevention preclinical trial was to test the efficacy of the COX-2 specific inhibitor *Celecoxib* to prevent the appearance and/or progression of DMBA-induced ovarian lesions. Most recently, the results of large clinical trials with this and other COX-2 specific inhibitors have demonstrated serious toxicities and side effects on the basis of which clinical trials have been put temporarily on hold. Because of the overall benefit of these agents, their testing will probably continue, however, we decided to postpone the proposed preclinical testing of *Celecoxib* in order to avoid the possibility of obtaining results that may deem unrelevant for the clinic. Previously, in collaboration with Dr. S. K. Dey, we tested a number of rat ovarian samples containing DMBA-induced lesions of various degrees of neoplastic development, for the relative expression of COX-1 and 2. This is due to his recent observations that COX-1 but not COX-2 is frequently overexpressed in human ovarian cancers (3). The results from this collaborative study strongly suggest that COX-1 protein is also present in the rat ovarian lesions at relatively higher levels than COX-2, and more importantly, contrary to COX-2, elevated expression of COX-1 is observed both in putative preneoplastic and neoplastic lesions. Based on these results, we opted to test a COX-1 specific inhibitor as a potential chemopreventive agent for ovarian cancer development (15). SC-560, available from Cayman Chemical Co, is orally active in the rat, where 10mg/kg completely abolishes the ionophore-induced production of thromboxane B2 in whole blood. This agent can be administered to animals via drinking water (15) in a preclinical chemoprevention trial with the rat DMBA model. However, due to change of PI and closure of the DMBA/gonadotropin animal protocol pertaining to this project, the proposed COX inhibitor chemoprevention trial in rats has not been initiated. Therefore, specific aims 2 and 3 relating to the project are not being carried out.

KEY RESEARCH ACCOMPLISHMENTS

The following are the key research accomplishments during the course of this DoD-CDMRP grant:

- Completion of first DMBA ovarian carcinogenesis experiment and collection of all rat ovarian tissues.
- Completion of histopathological analysis of all ovaries harvested from the above experiment and selection of ovaries harboring lesions; lesion classification according to previously described lesion categories.
- Statistical analysis of obtained data confirming the role of gonadotropin hormones as promoters of ovarian cancer development.
- Collection of the epithelial component of lesions from all selected ovaries, as well as normal OSE samples, by LCM.
- Purification and extensive quantitative and qualitative analysis of total RNA from LCM-derived sample; RNA subjected to two round of amplification and assessed for quantity and quality, prior to microarray analysis.
- Microarray analysis of rat ovarian lesions is currently underway.
- IHC analysis indicated a strong association of COX-1, and to a lesser degree COX-2 elevated expression with ovarian cancer development in the DMBA model.
- The observed frequent loss of the A20 ubiquitin-editing enzyme in human ovarian cancer may represent one of the key mechanisms leading to elevated, persistent activation of NF-κB and the development of platinum chemoresistance. Based on the findings from human samples we expect A20 to be expressed in normal rat OSE and lost in the neoplastic lesions.

REPORTABLE OUTCOMES

None

CONCLUSIONS

We have shown that administration of gonadotropin hormones in rats leads to the development of ovarian epithelial lesions of putative preneoplastic nature that resemble those appearing in ovaries of animals exposed to DMBA alone or DMBA/gonadotropins. This result, as well as the observed statistically significant increase in ovarian tumor incidence and malignant progression in animals treated with DMBA/gonadotropin versus DMBA alone, direct application of a low dose of DMBA conclusively demonstrate that gonadotropin hormones promote ovarian cancer development. We have also shown that the protein expression of COX-1, and to a lesser degree COX-2, is significantly increased in putative preneoplastic and neoplastic ovarian lesions induced by DMBA or DMBA/gonadotropins. Given that elevated COX-1 expression has been previously shown to be associated also with human ovarian cancers, it is reasonable to test the efficacy of the COX-1 specific inhibitor SC-560 to prevent the development of ovarian cancer using the DMBA/gonadotropin animal model. Recently, our microarray-based genomic analysis of primary human ovarian cancer specimens revealed that the expression of the dual ubiquitin-editing enzyme A20, a key regulator of NF-κB activation, is lost during ovarian cancer development. This conclusion is based on the fact that A20 mRNA expression, which is detected

at a moderate level in normal human OSE cells (cultured or not), is below reliably detectable levels in all ovarian tumor specimens tested, regardless of histological subtype or stage of malignancy. Hence, loss of A20 may represent an early, confounding event in ovarian oncogenesis, and may be associated with the frequently observed increased, persistent activation of NF-κB, and potentially with the development of resistance to platinum-based chemotherapy. In our current microarray analysis of the rat ovarian lesions, we will examine the status of genes that are known targets of DMBA mutations, as well as genes that may specifically apply to human ovarian cancer, such as p53, K-Ras, and c-Myc, ER and PR receptors, HER2/neu, Akt2, Bcl-2, Bcl-x and Bax. Moreover, we expect to identify genes whose changes in expression are associated with increased ovarian lesion severity and malignant progression, from nonneoplastic and preneoplastic to neoplastic.

REFERENCES

1. Stewart SL, Querec TD, Ochman AR, Gruver BN, Bao R, Babb JS, et al. Characterization of a carcinogenesis rat model of ovarian preneoplasia and neoplasia. Cancer Res. 2004 Nov 15;64(22):8177-83.
2. Ting AY, Kimler BF, Fabian CJ, Petroff BK. Characterization of a preclinical model of simultaneous breast and ovarian cancer progression. Carcinogenesis. 2007 Jan;28(1):130-5.
3. Gupta RA, Tejada LV, Tong BJ, Das SK, Morrow JD, Dey SK, et al. Cyclooxygenase-1 is overexpressed and promotes angiogenic growth factor production in ovarian cancer. Cancer Res. 2003 Mar 1;63(5):906-11.
4. Heyninck K, Beyaert R. A20 inhibits NF-kappaB activation by dual ubiquitin-editing functions. Trends Biochem Sci. 2005 Jan;30(1):1-4.
5. Wertz IE, O'Rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S, et al. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. Nature. 2004 Aug 5;430(7000):694-9.
6. Aghajanian C. Clinical update: novel targets in gynecologic malignancies. Semin Oncol. 2004 Dec;31(6 Suppl 16):22-6; discussion 33.
7. Perlmutter MA, Best CJ, Gillespie JW, Gathright Y, Gonzalez S, Velasco A, et al. Comparison of snap freezing versus ethanol fixation for gene expression profiling of tissue specimens. J Mol Diagn. 2004 Nov;6(4):371-7.
8. Wang SS, Sherman ME, Rader JS, Carreon J, Schiffman M, Baker CC. Cervical tissue collection methods for RNA preservation: comparison of snap-frozen, ethanol-fixed, and RNAlater-fixation. Diagn Mol Pathol. 2006 Sep;15(3):144-8.
9. Su JM, Perlaky L, Li XN, Leung HC, Antalffy B, Armstrong D, et al. Comparison of ethanol versus formalin fixation on preservation of histology and RNA in laser capture microdissected brain tissues. Brain Pathol. 2004 Apr;14(2):175-82.
10. Kabbarah O, Pinto K, Mutch DG, Goodfellow PJ. Expression profiling of mouse endometrial cancers microdissected from ethanol-fixed, paraffin-embedded tissues. Am J Pathol. 2003 Mar;162(3):755-62.
11. Gillespie JW, Best CJ, Bichsel VE, Cole KA, Greenhut SF, Hewitt SM, et al. Evaluation of non-formalin tissue fixation for molecular profiling studies. Am J Pathol. 2002 Feb;160(2):449-57.
12. Tunca JC, Erturk E, Erturk E, Bryan GT. Chemical induction of ovarian tumors in rats. Gynecol Oncol. 1985 May;21(1):54-64.

13. Schena M, Shalon D, Heller R, Chai A, Brown PO, Davis RW. Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. Proc Natl Acad Sci U S A. 1996 Oct 1;93(20):10614-9.
14. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci U S A. 1998 Dec 8;95(25):14863-8.
15. Daikoku T, Wang D, Tranguch S, Morrow JD, Orsulic S, DuBois RN, et al. Cyclooxygenase-1 is a potential target for prevention and treatment of ovarian epithelial cancer. Cancer Res. 2005 May 1;65(9):3735-44.

APPENDICES

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